transient transfection experiments, respectively. The role of the PGD_2 receptors, D prostanoid receptor 1 (DP1) and chemoattractant-receptor-like molecule expressed on Th2 cells (CRTH2), was evaluated using specific agonists and antibody blocking experiments. The contribution of the cAMP/PKA pathway was determined using cAMP elevating agents and PKA inhibitors.
Results: PGD_2 dose-dependently decreased Il-1-induced MMP-1 and MMP-13 protein and mRNA expression as well as MMP-1 and -13 promoter activation. DP1 and CRTH2 are expressed and functional in chondrocytes. The effect of PGD_2 was mimicked by the selective DP1 agonist BW245C, but not by the CRTH2 selective agonist DK-PGD_2. Furthermore, treatment with an anti-DP1 antibody reversed the effect of PGD_2, indicating that the inhibitory effect of PGD_2 is mediated by DP1. The cAMP elevating agents, 8-Br-cAMP and forskolin, suppressed Il-1-induced MMP-1 and MMP-13 expression, and the PKA inhibitors, KT5720 and H-89, reversed the inhibitory effect of PGD_2, suggesting that the effect of PGD_2 is mediated by the cAMP/PKA pathway.
Conclusions: PGD_2 inhibits Il-1-induced MMP-1 and MMP-13 production by chondrocytes through the DP1/cAMP/PKA signalling pathway. This suggest that modulation of PGD_2 levels in the joint may have therapeutic potential in the prevention of cartilage degradation.

186 "EX VIVO" ANALYSIS OF THE FIRST DOWNSTREAM EVENTS OF TGF-β SIGNALING IN HUMAN OSTEOARTHRITIC CARTILAGE

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Purpose: Some TGF-beta family members are critical players in cartilage homeostasis and repair. They act through phosphorylation of R-SMADs leading to activation of a series of downstream events. The precise regulation of SMADs remains unclear with the participation of both transcriptional and posttranslational mechanisms. The recent identification of a phosphatase, PPM1A, which specifically acts on SMADs has provided clues to understand termination of TGF-beta signaling. We aimed to study for the first time PPM1A in cartilage together with SMAD-2 and -3, the two SMADs that are phosphorylated in response to TGF-beta.
Methods: Cartilage samples were obtained from 11 patients with hip OA and 11 patients with femoral neck fracture that were undergoing total hip replacement. Gene expression of PPM1A, SMAD2 and SMAD3 was analyzed by RT-qPCR. Immunohistochemistry of the three proteins encoded by these genes and of the phosphorylated forms of Smad2 and Smad3 was performed.
Results: PPM1A was found to be expressed in both, OA and control human cartilage. SMAD2 expression was slightly increased in OA cartilage ($p = 0.04$) in relation with control cartilage, whereas no significant difference was found in PPM1A and SMAD3. No significant correlation was found between the expression of any of the three molecules. Preliminary immunostaining results showed no marked differences between OA and control cartilage.
Conclusions: Demonstration of PPM1A in human cartilage indicates that this phosphatase is also involved in TGF-beta signal regulation in this tissue. However, it was not differentially regulated in OA. SMAD2 expression was slightly higher in OA than in control cartilage indicating the need to study its regulation and suggesting the possibility of enhanced TGF-beta downstream signaling in OA. However, a more extensive analysis of Smad2 and Smad3 phosphorylation should be done before reaching a sound conclusion.

187 INTRACELLULAR OXIDANT PRODUCTION BY CHONDROCYTES IN OSTEOCHONDRAL EXPLANTS FOLLOWING BLUNT IMPACT INJURY

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Purpose: Acute chondrocyte necrosis and apoptosis induced by mechanical injury to articular cartilage is thought to contribute to the pathogenesis of post-traumatic osteoarthritis (PTOA). Ongoing work in our laboratory indicated that injury-induced chondrocyte death could be significantly inhibited by the free radical scavenger n-acetyl cysteine (NAC). This finding suggested that damaging levels of reactive oxygen species (ROS) were produced by chondrocytes in response to mechanical injury. An in vitro cartilage injury model was used to test this hypothesis. Osteochondral explants injured by high-energy blunt impact loading were stained with dihydroethidium (DHE) to probe for superoxide production by chondrocytes. Intracellular oxidation of DHE resulting in the accumulation of ethidium in oxidant-producing cells was monitored by confocal microscopy. Further support was provided by an NADPH-oxidase (NOX) inhibitor, which reduced chondrocyte death post-blunt impact.
Methods: To simulate cartilage injuries sustained in vivo as a result of blunt trauma we subjected bovine osteochondral explants to a single blunt impact blow (2.5 or 5 J) via a 5 mm diameter platen using a drop tower device. Imaging of superoxide production was accomplished by incubating the explants in complete medium (DMEM, F-12, 10% Fetal Bovine Serum) equilibrated in low oxygen conditions with 5 μM DHE.

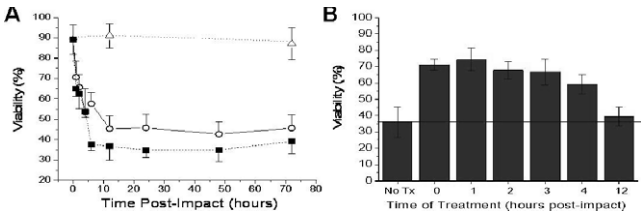

Figure 1. Time course of post-impact chondrocyte death and NAC effects. Superficial viability of chondrocytes. A illustrates the differences in viability across time between two different impact energy levels (circle = 2.5 J, square = 5 J, triangle = control). B illustrates the viability when treated post-impact with NAC (48 hours post-2.5 J).

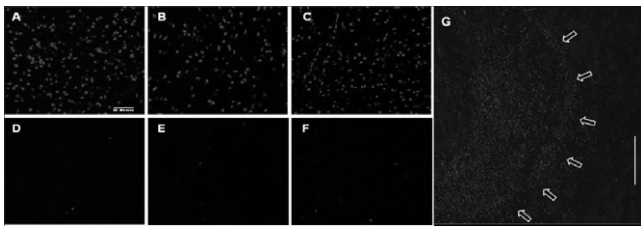

Figure 2. Impact-induced ROS production in osteochondral explants. The images show Z-axis reconstructions of DHE-stained chondrocytes (bright red nuclei) in impacted cartilage (A,B,C) and in adjacent non-impacted cartilage (D,E,F) at 30 minutes (A,D), at 60 minutes (B,E), and at 2 hours (C,F) post-injury. The bar in A is 100 μm long. (G) Low magnification imaging (bar = 1 mm) revealed heavy accumulation of DHE-stained chondrocytes within and closely adjacent to the border of the circular impact site (arrows) at 2 hours post impact.

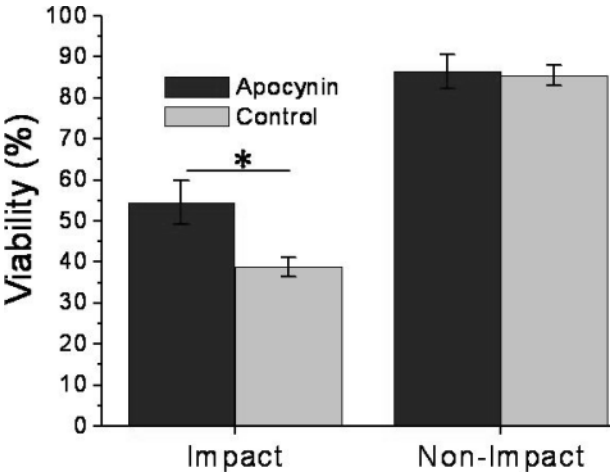

Figure 3. The NOX inhibitor apocynin inhibits impact-induced chondrocyte death. Explants cultured with or without apocynin for 3 days were subjected to a 2.5 J impact (Impact). A set of controls were cultured for 3 days but were not impacted (Non-Impact). Columns and error bars show means and standard deviations based on 3 explants. The bar and asterisk indicate a significant difference between apocynin-threated and control.

Results: Impact injuries to osteochondral explants cause chondrocyte death and a localized production of superoxide radicals. Previously, a time-course for post-impact chondrocyte viability was determined for two different energy levels (2.5 and 5 J) and treatment with NAC within 4 hours of impact injury spared significant numbers of chondrocytes (Figure 1). DHE staining revealed a dramatic accumulation of ethidium in chondrocytes within 30 minutes of impact injury, indicating increased superoxide production (Figure 2). Apocynin, an inhibitor of NADPH-oxidase (NOX) reduced chondrocyte death induced by impact (Figure 3).
Conclusions: Our data support the hypothesis that superoxide production is initiated by chondrocytes after mechanical trauma and suggest chondrocyte death results from superoxide release. The observation that apocynin blocked impact induced death suggests that NOX is a source of superoxide production post-injury. The next step in these studies will be to test various inhibitors of NADPH oxidase and other potential sources of ROS for effects on free radical production and chondrocyte death. Such inhibitors may be used to augment the cell-sparing effects of NAC, a strategy that has the potential to forestall the onset of PTOA.

188 MORPHOLOGICAL DESCRIPTION OF THE ANATOMY OF CERVICAL SPINE FACET JOINTS BY FACET ORIENTATION

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Purpose: The purpose of this paper is to describe the morphology of the lower cervical spine facet joints according to facet orientation in a young population.
Methods: The cervical spine segments (C4-C7) from 40 subjects were removed during autopsy (12 females and 28 males, median age 35 years, range 20−49). Twenty-one subjects had died due to non-traumatic causes and 19 following a motor vehicle collision. Each specimen was fixed in alcohol and embedded in methylmethacrylate. Each specimen was divided into 3-mm thick parasaggital slices from which 10 μm thick histological sections were produced and stained with Masson-Goldner trichrome. A total of 636 unique facets were examined microscopically with regard to cartilage defects such as vertical fissures (any size and complete (i.e. 100%)), horizontal splitting, and superficial fibrillation (flaking), vascular invasion, and osteophytes were evaluated. Each variable was evaluated for correlation to the orientation of the articular facets (superior or inferior), and the contributions from gender and exposure to trauma. The Chi^2-test was used for testing of the association of groups, using STATA© 9.2 software. The significance level was $p < 0.05$. The potential influence of effect modification from the exposure to trauma, gender and age was not evaluated.

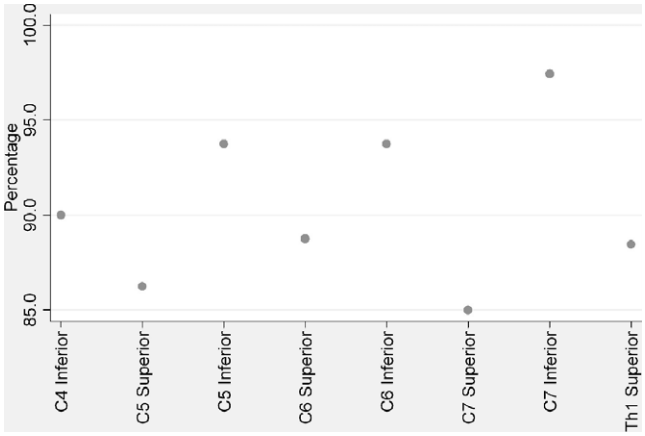

Figure 1. Superficial fibrillation by facet level.

Results: Fissures of any size were identified in 80% of the superior and inferior facets with no difference between the facets. Males were more often affected by fissures, in both facet orientations, in comparison to females ($p < 0.01$). Complete vertical fissures were identified in 6% of all facets, and was more common overall in the superior facets ($p < 0.05$), and males were more often affected in the inferior facets ($p < 0.05$). Horizontal splitting was present in 90% of all facets and did not show any difference between the facet orientation overall. However, stratifying the data with regard to the contribution from gender and trauma, males were found to have splitting more often than females in both facet orientations ($p < 0.001$), and trauma cases were more often affected ($p < 0.05$). Superficial fibrillation was present in 90% of the facets, and males were more likely to be affected in both the superior ($p < 0.001$), and inferior facets ($p < 0.01$) (Figure 1). Furthermore, the inferior facets were more severely affected by these changes ($p < 0.01$). Vascular invasion was identified in 33% of the facets with no differences between the facets overall, although males were generally more affected, in particular the inferior facets ($p < 0.01$). Osteophytes were present in 8% of the facets and were not influenced by orientation or gender. Neither superficial fibrillation, fissures of any kind, vascular invasion nor osteophytes was affected by the exposure to trauma.
Conclusions: This study of the cervical spine facets joints in a young population described the prevalence of a number of morphological variables with regard to facet orientation. Disorders of the cartilage such as superficial fibrillation, vertical fissures and horizontal splitting were all common. Overall, the inferior articular facets seemed to be more affected in comparison to the superior counterpart, and males were more often affected. Only horizontal splitting of the cartilage was correlated to trauma. The findings may have relevance to clinical practice where early degenerative changes in the cartilage can predispose to cervical spine pain syndromes, and influence the loading capabilities of the facet joints during traumatic as well as normal physiological stresses.

189 RECOGNITION OF ALTERNATIVELY SPLICED FIBRONECTIN ISOFORMS BY A RAT CHONDROCYTE CELL LINE AND ITS MESENCHYMAL STEM CELL PRECURSOR LINE

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Purpose: The balance between chondroprogenitor cells and differentiated articular chondrocytes may play a role in the progression of osteoarthritis (OA). Elucidation of differences in signaling between these two types of cell will enhance our understanding of how mesenchymal stem cells and differentiated chondrocytes interact with each other and with the ECM in healthy versus OA cartilage.
Methods: We investigated differences in integrin-mediated recognition of different alternatively spliced fibronectin (FN) variants in a rat chondrocytic cell line, RCJ 3.1C5.18, and its mesenchymal progenitor cell line, RCJ 3.1. Cell adhesion assays were used to determine the nature of integrin-mediated cell recognition of different FN splice variants. FN variants were expressed as GST and/or His-tagged fusion proteins.
Results: In the presence of 1 mM Ca++ and 1 mM Mg++, both cell lines recognized the alternatively spliced V segment and the 10th type III repeat of FN, both of which contain RGD sequences. In contrast, it was necessary to include Mn++ in the cell medium in order for the isolated EIIIA segment of FN to be recognized by either line. Recognition of the EIIIA segment was specific, since pretreatment of protein-coated assay wells with a monoclonal antibody (MAb) specific for the EIIIA segment blocked adhesion by both cell lines. Also, Mn++-dependent recognition of EIIIA by C5.18 cells was blocked by pretreatment of the cells with b1 integrin function-blocking MAb, while recognition of EIIIA by the parental cell line was blocked by both b1 and av-blocking MAbs. Half-maximal binding of 3.1 cells to the isolated V and III-10 segments decreased compared to the C5.18 cells.
Conclusions: We demonstrated that a mesenchymal stem cell line and its chondrocytic progeny line both recognized the alternatively spliced V and EIIIA segments of FN. In both the parental and progeny cell lines, recognition of the V segment did not require integrin activation by Mn++, whereas recognition of the alternatively spliced EIIIA did. Antibody blocking experiments revealed that the parental cell line required both av and b1 subunits for recognition of the EIIIA segment, whereas such recognition was only blocked by anti-b1 MAbs in the progeny line. Together, these results suggest that different cell signaling pathways are triggered by FN splice variants depending on the state of cell differentiation. Further studies should investigate what signaling molecules are involved inside the cell and what effect these have on chondrocyte differentiation/dedifferentiation in osteoarthritic articular cartilage.